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Quarterly Reports

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The 17th ISFR Conference will be held in Budapest, Hungary from June 23 through June 25, 1989 in the Sporthall, which is centrally located. The official language will be English.

The meeting, which will be organized by **Dr. Miklós Bély**, will stress problems with fluoride: chemistry; toxicology; biological effect; instruments and measuring techniques; effect of fluoride on plants, animals, humans; osteofluorosis.

It will be held as a separate conference but at the same time as one sponsored by the Hungarian Society for Rheumatism, which will bring specialists from Hungary, Poland, West and East Germany. The Hungarian Society sponsors and covers costs thanks to the President of the Society, Prof. Dr. Béla Gömör.

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THE DECLINE IN PRIMARY TOOTH DECAY IN NEW ZEALAND BEFORE THE USE OF FLUORIDES*

SUMMARY: Official data collected in New Zealand over a 50-year period suggest that the general decline in decay of primary teeth in 5-year-olds started well before the wide-spread use of fluoride and is closely related to the expansion of school dental services.

The dramatic decline in dental decay which has occurred in recent decades in developed countries (1), including New Zealand (2), has been attributed to water fluoridation and the increased use of fluoride. However, recent commentaries have drawn attention to temporal reductions in both primary (3) and permanent (4) tooth decay, which cannot be attributed to fluoridation or fluorides. In Australia and New Zealand differences in dental decay prevalence between fluoridated and non-fluoridated areas are small (4-9). It has been suggested that diet and immunity are factors which contributed to these declines (4). J.M. Dunning has suggested another factor: ". . . caries is now recognized as an infectious disease. Perhaps it is also contagious, and the sum total of our preventive measures, to date, may have so reduced the bacterial reservoir that channels of contagion are drying up." (10) Regular dental treatment is one of the measures which reduces that reservoir.

In New Zealand children commence schooling at 5 years of age. For this age group dental health statistics, which have been continuously kept, provide a valuable epidemiological record (11-17). Starting in 1922 the New Zealand School Dental Service gradually expanded, raising the age of those enrolled, with pre-school enrollments also increasing until, by 1965 almost all school-children aged 5 to 12 or 13 years, and most preschool children, were receiving regular dental care at school clinics (11). Primary as well as permanent teeth have been treated at 6-monthly intervals, increasingly by fillings rather than extractions, so that eventually almost all children have experienced early removal of the diseased tissue which harbored the "bacterial reservoir." Counselling on diet and oral hygiene, likewise to mothers of preschool children, accompanied such treatment.

Data were collected from the following sources: 1) from 1940 to 1971 Department of Health information on all 5-year-old new patients as they enrolled in the School Dental Service (11); 2) An early study of primary teeth from 1932 to 1948-50 in the Wellington region (12) where, according to a 1948-50 national survey (13), decay prevalences did not differ from the national average; 3) Recent studies in 1977 and 1982 (14,15); 4) Official information on the growth of the School Dental Service (11); 5) Official information on the introduction of water fluoridation (16). The information is presented in the Figure.

The Figure shows that improvement had started well before the introduction of water fluoridation. Even if one questions the 1932 information, after 1940 a decay decline was clearly underway. In the 1960's other fluoride uses (tablets and clinical applications) became widespread about the same time as water fluoridation, but fluoride toothpastes were not widely marketed until

Presented in part at the 15th Conference of ISFR, July 31-August 2, 1986 at Utah State University, Logan, Utah, USA



Decline in Dental Decay of 5-Year-Olds. Solid line: Average no. decayed, missing and filled teeth (dmft). Broken line: Dental decay prevalence (100 percent caries-free).

Enrollment in School Dental Service. Broken Line: percent of total child population. Solid line: percent of pre-school population.

Fluoride source. Solid line: percentage of population with water fluoridation. Broken line: fluoride toothpaste percentages of total toothpaste sales.

Date of Collection	Size of Sample	dmft	Percent caries-free	Source reference	
1930-32	263	11.2	0.7%	12	
1940	"70 Clinics"	8.48	4.35%	11	
1948-50	692	7.1	12.28%	12	
1950	22,514	7.45	-	11	
1950	"70 Clinics"	6.85	14.37%	11	
1955	44,976	7.34	-	11	
1960	"70 Clinics"	6.07	16.74%	11	
1961	65,001	5.87	-	11	
1966	87,499	5.17	-	11	
1971	88,573	4.04	-	11	
1977	998	3.75	34%	14	
1982	958	2.6	44%	15	

DENTAL DATA AND SOURCES

For any one year dmft for the larger or largest sample is presented in the Figure: 4000 to 5000 new 5-year-old patients attended "70 clinics."

Editorial

well after 1970 (16). Thus, fluoride could only have accelerated the last stage of the decline in decay of primary teeth, which has continued in both non-fluoridated and fluoridated areas (9,14,15). However, this decline and the gradual expansion of the School Dental Service are well correlated.

Similar continuous statistical information is not available in New Zealand for older children and, therefore, for permanent teeth. However, a similar overall decline in permanent tooth decay, with or without fluoridation, has occurred between 1950 and today (9,17). This decline was much steeper, in both fluoridated and non-fluoridated areas, after 1977, the year of a significant national change in diagnostic procedure within the School Dental Service (5,6). Since the disease process for carles of permanent teeth is not essentially different from that of primary teeth, it seems probable that a similar decline in permanent tooth decay could have occurred in other developed countries independently of fluoridation.

Evidence for the early decline in primary tooth decay, based on the dental data (1932-55) then available, was presented in 1957 to the New Zealand Commission of Inquiry into fluoridation by the Director of the School Dental Service. He attributed the decline to education about diet and oral hygiene among mothers and young children (18). Nevertheless both he and the Commission believed that water fluoridation would be the only effective way to reduce the dental decay still rampant among older children. Few, at that time, appear to have suspected that the above trend in the lower age group might be the start of a decline which would continue and eventually affect the older age groups as well. Research underway shows that by 1981, a great improvement in the average New Zealand diet had occurred; more protective foods were being eaten, including more than double the 1930 consumption of fresh fruit and vegetables (summary supplied by Professor J.A. Birkbeck, N.Z. Nutrition Foundation).

In 1977, Professor D.J. Beck made the following comment on the data on 5-year-olds: "It is certainly not a fluoride effect, being too early for that - at least between 1940 and 1950. Perhaps it is my turn to be naive when I ascribe the improvement to health education . .." (19). The current official view is: "The Division of Dental Health believes that the decline in caries prevalence in the deciduous dentition from 1950 to the early 1970's was largely a result of the increasing affluence and increasing general education of the population. The decline in caries prevalence since the early 1970's (which is more marked in the permanent rather than the deciduous dentition) is believed to be related to the increased availability of fluorides in tablet form and, in particular, in toothpaste, and fluoridation, and a change to a preventive orientation in the School Dental Service" (20). However, the evidence presented in this paper suggests that child dental health in New Zealand, as in Denmark (21), has been significantly influenced by the continuing educational, preventive and early treatment approach to the problem, rather than by fluoridation.

References

- 1. Sheiham, A.: Changing Trends in Dental Caries. Int. J. Epidemiol., 13:142-147, 1984.
- 2. Brown, R.H.: Evidence of Decrease in the Prevalence of Dental Caries in New Zealand. J. Dent. Res., 61(Special Issue):1327-1330, 1982.

Editorial

- Jackson, D.: Has the Decline in Dental Caries in English Children Made 3. Water Fluoridation Both Unnecessary and Uneconomic? Br. Dent. J., 162:170-173, 1987.
- Diesendorf, M.: The Mystery of Declining Tooth Decay. Nature, 322:125-4. 129, 1986.
- de Liefde, B., and Ritchie, G.R.: Evaluation of Dental Public Health in 5. New Zealand, N.Z. Dent. J., 80:8-14, 1984.
- Hunter, P.B.V.: The Prevalence of Dental Caries of 12- and 13-Year-Old 6. New Zealand Children in 1977 and 1982. N.Z. Dent. J., 80:16-18, 1984.
- 7. Colquhoun, J.: Influence of Social Class and Fluoridation on Child Dental Health. Community Dent. Oral Epidemiol., 13:37-41, 1985.
- Colquhoun, J.: Child Dental Health Differences in New Zealand. Community 8. Health Studies, 11:85-90, 1987.
- Annual Report. New Zealand Department of Health. Government Printer, 9. Wellington, 1985, pp. 26-27, 69.
- 10. Dunning, J.M.: Commentary on "New Evidence on Fluoridation." Soc. Sci. Med., 19:1244-1246, 1984.
- 11. Annual Reports, New Zealand Department of Health. Government Printer, Wellington, 1957-1984.
- 12. Hewat, R.E.T., Eastcott, D.F., and Bibby, J.B.: The Prevalence of Dental Caries in the Deciduous Teeth of New Zealand Children. N.Z. Dent. J., 48:160-173, 1952.
- 13. Hewat, R.E.T., and Eastcott, D.F.: Dental Caries in New Zealand. Medical Research Council of New Zealand, 1953.
- 14. Hunter, P.B.V.: The Prevalence of Dental Carles in 5-Year-Old New Zealand Children. N.Z. Dent. J., 75:154-157, 1979. 15. Hunter, P.B.V.: The Prevalence of Dental Caries in 5-Year-Old New
- Zealand Children in 1977 and 1982. N.Z. Dent. J., 80:14-16, 1984.
- 16. Dental Division Files, 1982-1985. Department of Health, Wellington, N.Z.
- 17. Fulton, J.T.: Experiment in Dental Care. Results of New Zealand's Use of School Dental Nurses, Monograph No. 4. World Health Organization, Geneva, 1951.
- 18. Report of Commission of Inquiry on the Fluoridation of Public Water Supplies. Government Printer, Wellington, 1957, p. 23.
- 19. Beck, D.J.: Dental Health Education and Preventive Measures, Success or Failure? In Oral Health Needs of the Adult Population. Brown, R., ed. Proceedings of a Symposium, Univ. of Otago, Dunedin, N.Z., 1977.
- 20. Letter of Department of Health to N.Z. Government Toxic Substances Board, July 11, 1985.
- 21. Billie, J., Hesselgren, K., and Thylstrup, A.: Dental Caries in Danish 7-, 11- and 13-Year-Old Children in 1963, 1972 and 1981. Caries Res., 20:534-542, 1986.

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LIVER AND LUNG ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN BENZO(a)PYRENE TREATED RATS: LACK OF EFFECT OF HYDROGEN FLUORIDE

by

G. Bompart*, J. Rakotoarivony and Y. Manuel Toulouse Cedex, France

SUMMARY: We have studied the effect of benzo(a)pyrene (B(a)P) and hydrogen fluoride (HF), given separately or together to rats on (a) the liver and lungs aryl hydrocarbon (B(a)P) hydroxylase activity and (b) cytochrome P_{450} levels in liver and lungs. B(a)P was administered intraperitoneally once a week and HF by continuous inhalation, over a total period of 157 days.

The results show a high aryl hydrocarbon hydroxylase activity induced by B(a)P in the lung microsomes; however, HF does not exhibit any effect. The slight increase of lung cytochrome $P_{4.5.0}$ levels induced by B(a)P is not significant. On the other hand, B(a)P and HF do not induce any effect on aryl hydrocarbon hydroxylase activity and cytochrome $P_{4.5.0}$ levels in the liver.

KEY WORDS: Aryl hydrocarbon hydroxylase; Benzo(a)pyrene; Cytochrome P450; Hydrogen fluoride; Microsomes; Polycyclic aromatic hydrocarbon.

Introduction

Benzo(a)pyrene (B(a)P) is a procarcinogen polycyclic aromatic hydrocarbon present in our environment. In the cells, it is metabolized by the microsomal aryl hydrocarbon hydroxylase into many derivates including epoxides, phenols, and quinones (1,2). Epoxides are transformed into diols by epoxide hydrolase and the diols are recycled through the aryl hydrocarbon hydroxylase to yield diol epoxides which are generally considered to be the ultimate carcinogens (3-6).

The activity of these enzymes depends on various compounds. Gelboin (2) demonstrated a relationship between the enzymatic activity of aryl hydrocarbon hydroxylase and the cytotoxic effects of polycyclic aromatic hydrocarbons. The data indicated that the inhibition of aryl hydrocarbon hydroxylase by 7-8-benzo-flavone decreases the toxic effect of polycyclic aromatic hydrocarbons (7,8) by preventing the covalent binding of nucleic acids and protein (9). Gorban (10) found that sodium fluoride incorporated into the diet of rats at a daily dose of 1.5 mg F/kg reduced the rate of liver tumors induced by p-dimethyl-aminoazobenzene. Palmer (11) showed an inhibition of benzopyrene hydroxylase activity by several concentrations of O_{3x} and no demonstrable early or delayed effect of NO₂ inhalation.

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Since HF and polycyclic aromatic hydrocarbons are commonly present together in industrial atmospheres, this study was undertaken to investigate (a) whether repeated administrations of benzo(a)pyrene can have an effect on aryl hydrocarbon hydroxylase activity and cytochrome P_{*50} levels in lungs and liver; and (b) whether HF can have a measureable effect on these parameters. Moreover, since aryl hydrocarbon hydroxylase is an isoenzyme of the cytochrome P_{*50} system, we have measured the total CO-binding cytochrome P_{*50} in order to investigate whether the modifications observed in aryl hydrocarbon hydroxylase activity can be correlated with the total cytochrome P_{*50} .

Materials and Methods

Exposure room. Male Sprague-Dawley rats of 250 g average weight, housed six per cage, were exposed 24 hours each day to HF in an air-conditioned room (volume 20 m³, temperature 20°C, humidity 75%) with an air turnover rate of 160 m³/h at a level of 0.60 \pm 0.15 mg HF/m³. Such HF concentration was chosen because it is commonly encountered in industrial atmosphere (aluminum, fertilizers) and in various experimental designs (12,13). Gaseous HF was produced from an aqueous HF solution transferred by a peristaltic pump into a vaporization PTFE oven thermostated at 150°C. The desired concentration of atmospheric HF was obtained by adjusting the concentration and the flow of the aqueous HF solution, whereas the flow of air prealably purified by passing through a cellulose filter impregnated with sodium hydroxide was constant. The atmospheric concentration was checked daily by passing a measured volume of air through a cellulose filter impregnated with sodium hydroxide. The collected F was determined (14,15) by a fluoride specific electrode (Orion 96-09). During exposure, the rats had free access to water and food.

Experimental design and techniques. The rats were segregated into four groups of 60 animals as follows: a control group which received a weekly intraperitoneal injection of 0.25 mL/rat of vehicle (sunflower oil); an HF group treated as the control was constantly exposed to an HF atmosphere; a B(a)P group was treated weekly with 2.5 mg B(a)P until the end of the experiment; an HF + B(a)P group was treated with both B(a)P and HF as described above.

Five rats of each group were killed on the third day and every fortnight thereafter. Blood was drained by aortic puncture, following which ionic fluoride was determined using a fluoride specific electrode. Liver and lungs were removed and homogenized in a 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged twice at 10,000 g for 10 min. After separation the supernatant was again centrifuged at 100,000 g for 1 h. In the microsomal fraction, the protein was determined using Lowry's method (16); cytochrome P_{450} levels and aryl hydrocarbon hydroxylase (benzopyrene hydroxylase) activity were quantified usine McLean (17) and Nebert-Gelboin's (18) method respectively. Cytochrome P_{450} levels and aryl hydrocarbon hydroxylase activity were expressed as nmoles cyto P_{450}/mg of protein and nmoles 3-hydroxybenzopyrene produced/mg of protein/min.

Student's t-test was used to determine statistical significance. A p value of 0.05 or less was considered statistically significant.

Results and Discussion

Activity of pulmonary aryl hydrocarbon hydroxylase. For both control and HF groups, the pulmonary aryl hydrocarbon hydroxylase activity was lower than

Volume 21, No. 1 January, 1988 that of the liver by about a hundred fold. Figure 1 shows HF does not change the aryl hydrocarbon hydroxylase activity. On the other hand, in the two B(a)P treated groups, the aryl hydrocarbon hydroxylase activity increases rapidly and a plateau occurs at the 73rd day. In all these experiments, the presence of HF does not significantly influence (p > 0.05) the activity of the aryl hydrocarbon hydroxylase.



Figure 1

Aryl Hydrocarbon Hydroxylase (A.H.H.) Activity of Pulmonary Microsomes

Values are means of five determinations ($\overline{X} \pm S.D.$).

The strong inducibility of the aryl hydrocarbon hydroxylase may account for the high sensitivity of the lungs to the risk of cancer induced by polycyclic aromatic hydrocarbons. Genetic control of aryl hydrocarbon hydroxylase inducibility has been reported in mouse (19) and man (20,21). Whitlock (22) and Busbee (23) showed that aryl hydrocarbon hydroxylase is inducible in the human cultured lymphocytes and leukocytes systems. Later Kellermann (24) reported that a group of lung cancer patients showed a higher level of inducible aryl hydrocarbon hydroxylase than a healthy control population. Kellermann inferred that an assessment of the risk of lung cancer might be possible, using individual levels of aryl hydrocarbon hydroxylase inducibility as the criterion for risk determination.

Activity of liver aryl hydrocarbon hydroxylase. Figure 2 does not show any



Values are means of five determinations (\overline{X} ±S.D.).

significant effect in the three groups of treated rats. The lack of B(a)P inductive effect can explain the low sensitivity of the liver for this carcinogenic compound. Thus Kitawaga (25) reported liver carcinoma caused by B(a)P only after partial hepatectomy and promotion by phenobarbital. On the other hand, Post and Snyder (26) reported that <u>in vitro</u> benzo(a)pyrene hydroxylation was not affected by fluoride.

<u>Cytochrome</u> P_{450} levels. For all groups, the pulmonary cytochrome P_{450} (Figure 4) was about ten fold lower than that of liver (Figure 3). These results are in agreement with those of other workers who report lung:liver cytochrome P_{450} ratios ranging between 10 (27) and 5 (28) percent.

The experiments carried out showed no effect of B(a)P, B(a)P + HF and HF on the liver cytochrome P_{450} measured levels (Figure 3). The lungs show a slight but not significant (p > 0.05) increase of cytocrome P_{450} levels in B(a)P and B(a)P + HF groups (Figure 4). For lungs (despite the increase of aryl hydrocarbon hydroxylase activity), this observation can be explained by the occurrence of several types of cytochrome P_{450} , and increases in a single type may not yield detectable increases in the total CO-binding reduced cytochrome P_{450} measurements. Thus benzene treatment increases the metabolism of benzene, zoxazolamine, neoprontosil and p-nitrobensoate without increasing cytochrome P_{450} measured levels (29,30).

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Figure 3 Cytochrome Pass Levels of Liver Microsomes

Values are means of five determinations (\overline{X} ±S.D.).

<u>Plasma ionic F</u> levels. This determination allows us to establish whether the rise in plasma F concentration is parallel, during the experimentation period, in the two groups of animals exposed to HF, while they remain unchanged in non-exposed animals (Figure 5).

References

- 1. Yang, S.K., McCourt, D.W., Leutz, J.C. and Gelboin, H.V.: Benzo(a)pyrene Diol Epoxides: Mechanisms of Enzymatic Formation and Optically Active Intermediates. Science, 196:1199-1200, 1977.
- 2. Gelboin, H.V., Huberman, E. and Sachs, L.: Enzymatic Hydroxylation of Benzopyrene and its Relationship to Cytotoxicity. Proc. Nat Acad. Sci. (Wash), 64:1188, 1969.
- Kapitulnik, J., Levin, W., Conney, A.H., Yagi, H. and Jerina, D.M.: Benzo(a)pyrene 7,8-dihydrodiol is More Carcinogenic than Benzo(a)pyrene. Nature, 266:378-380, 1977.
- Nakanishi, K., Kasi, H., Cho, H., Harvey, R.G., Jeffrey, A.M., Jennette, K.W. and Weinstein, I.B.: Absolute configuration of a ribonucleic acid adduct formed in vivo by metabolism of benzo(a)pyrene. J. Am. Chem., Soc., 99:258-260, 1977





- 5. Yang, S.K., Gelboin, H.V., Trump, B.F., Autrup, H. and Harris, C.: Metabolism Activation of Benzo(a)pyrene and Binding to DNA in Cultured Human Bronchus. Cancer Res., 37:1210-1215, 1977.
- Baird W.M., Harvey, R.G. and Brookes, P.: Comparison of the Cellular DNA-bound Products of Benzo(a)pyrene with the Products Formed by the Reaction of Benzo(a)pyrene-4,5 oxyde with DNA. Cancer Res., 35:54-57, 1975.
- 7. Diamond, L. and Gelboin, H.V.: Alpha-naphtoflavone: An Inhibitor of Hydrocarbon Cytotoxicity and Microsomal Hydroxylase. Science, 166:1023, 1969.
- Gelboin, H.V., Wiebel, F. and Diamon, L.: Dimethylbenzanthracene Tumorigenesis and Aryl Hydrocarbon Hydroxylase in Mouse Skin: Inhibition by 7,8 benzoflavone. Science, 170:169, 1970.
- Kinoshita, N. and Gelboin, H.V.: The Role of Aryl Hydrocarbon Hydroxylase in 7,12 benzoflavone Inhibition of Tumorigenesis. Cancer Res., 32:1329, 1972.
- Gorban, G.P., Pliss, M.B., Karpilovakaja, E.D., Petrun, A.S., Svatkov, V.I. and Rubenick, B.L.: The Effect of Long-term Administration of Fluorine with Food on the Carcinogenesis and Biochemical Changes in the Liver of Rats. Vaprosy Pitanija Moska, 6:21-24, 1975.
- Palmer, M.S., Exley, R.W. and Coffin, D.L.: Influence of Pollutant Gases on Benzopyrene Hydroxylase Activity. Arch. Environ. Health, 25:439-442, 1972.
- 12. Rioufol, C.: Les effects de HF inhalé sur des cobayes après des exposition à long terme et de courte durée. Thèse pour le Doctorat d'État es-Sciences Pharmaceutiques, 116, Toulouse, France, 1981.
- Bourbon, P., Rioufol, C. and Lévy, P.: Relationships Between Blood, Urine and Bone F Levels in Guinea Pig After Short Exposures to HF. Fluoride, 17:124-131, 1984.
- 14. Fry, B.W. and Taves, D.R.: Serum Fluoride Analysis with the Fluoride Electrode. J. Lab. Clin. Med., 76:1020, 1970.
- Fuchs, C. et al.: Fluoride Determination in Plasma by Ion Selective Electrode: A Simplified Method for the Clinical Laboratory, Clin. Chem. Acta, 60:157, 1975.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.: Protein Measurement with the Phenol Reagent. The J. Biol. Chem., 193:265-275, 1951.
- 17. McLean, A.E.M. and Day, P.: The Use of New Methods to Measure: The Effect of Diet and Inducers of Microsomal Enzyme Synthesis on Cytochrome P_{*50} in Liver Homogenates and on Metabolism of Dimethylnitrosamine. Biochem. Pharmachol., 23:1173-1180, 1974.
- Nebert, D.W. and Gelboin, H.V.: Substrate Inducible Microsomal Aryl Hydroxylase in Mammalian Cell Culture. The J. Biol. Chem., 243:6242, 1968.
- Nebert, D.W., Goujon, F.M. and Gielen, J.E.: Aryl Hydrocarbon Hydroxylase Induction by Polycyclic Hydrocarbons: Simple Autosomal Trait in the Mouse. Nature (New Biol.), 236:107-110, 1972.
- Kellermann, G., Luyten-Kellermann and Shaw, C.R.: Genetic Variation of Aryl Hydrocarbon Hydroxylase in Human Lymphocytes. Amer. J. Hum. Genet., 25:347-351, 1973.
- 21. Atlas, S.A., Vessell, E.X. and Nebert, D.W.: Genetic Control of Interindividual Variations in the Inducibility of Aryl Hydrocarbon Hydroxylase in Cultured Human Lymphocytes. Cancer Res., 36:4619-4630, 1976.
- 22. Whitlock, J.P., Cooper, H.L. and Gelboin, H.V.: Aryl Hydrocarbon (Benzo-

pyrene) Hydroxylase is Stimulated in Human Lymphocytes by Mitogens and Benz(a)anthracene. Science, 177:618-619, 1972.

- 23. Busbee, D.L., Shaw, C.R. and Cantrell, E.T.: Aryl Hydrocarbon Hydroxylase Induction in Human Leukocytes. Science, 178:315, 1972.
- Kellermann, G., Shaw, C.R. and Luyten-Kellermann, M.: Aryl Hydrocarbon Hydroxylase Inducibility and Bronchogenic Carcinoma. New Engl. J. Med., 289:934-937, 1973.
- Kitawaga, T., Hirakawa, T., Ishikawa, T., Nemoto, N. and Takayama, S.: Induction of Hepatocellular Carcinoma in Rat Liver by Initial Treatment with B(a)P after Partial Hepatectomy and Promotion by Phenobarbital. Toxicology Letter, 6:167-171, 1980.
- Post, G.B. and Snyder, R.: Fluoride Stimulation of Microsomal Benzene Metabolism. J. Toxicol. Environ. Health, 11:799-810, 1983.
- Liherst, C.L., Mimnaugh, E.G. and Gram T.E.: Comparative Alterations in Extrahepatic Drug Metabolism by Factors Known to Affect Hepatic Activity. Biochem. Pharmac. 26:749-755, 1977.
- 28. Capdevila, J., Jakobsson, S.W., Jernström, B., Helia, O. and Orrenius, S.: Characterization of Rat Lung Microsomal Fraction Obtained by Sepharose 2B Ultrafiltration. Cancer Res., 35:2820-2829, 1975.
- Saito, F.U., Kocsis, J.J. and Snyder, R.: Effect of Benzene on Hepatic Drug Metabolism and Ultrastructure. Toxicol. Appl. Pharmac., 26:209-217, 1973.
- Gonasun, L.M., Witmer, C., Kocsis, J.J. and Snyder, R.: Benzene Metabolism in Mouse Liver Microsomes. Toxicol. Appl. Pharmac., 26:398-406, 1973.

NAF EFFICACY IN THE OTOSPONGIOTIC-OTOSCLEROTIC DISEASE Seven Series of Experiments

by

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SUMMARY: NaF is an effective therapy which acts on the enzymatic mechanism leading to cochlear deterioration. Its action is widely discussed.

The purpose of this paper is to demonstrate NaF effectiveness by means of:

1. Biochemical methods which consist of studying NaF action on the proteolytic activity of otospongiotic perilymph samples taken at the time of stapedectomies.

2. Experimental methods, either in vitro or in vivo (laboratory animals and human perilymph).

3. Enzymatic methods through multiple enzyme correlations.

4. Cytochemical methods in studying the Ca/P ratio in otospongiotic focl.

5. Radiological methods through polytomographic changes seen in the cochlear capsule before and after NaF therapy.

6. Labelling products, using Strontium 85.

7. Clinical studies on computerized data and clinical doubleblind placebo-controlled investigations.

These experiments indicate that sodium fluoride action is effective.

KEY WORDS: Cochlear otosclerosis, enzyme, Immune, NaF, Otosclerosis, Otospongiosis,

Methods and Materials

The action of NaF therapy in the otospongiotic-otosclerotic disease has been widely discussed in the past, some have denied any activity of NaF on cochlear deterioration; others were confident in its efficacy. In spite of numerous studies, it is still controversial.

However, our numerous experiments in vitro, in vivo, on laboratory animals and on human patients through enzymatic, cytochemical and anatomo-pathological experiments, have led us to believe that the mechanism of NaF activity is an enzymatic action on the first phase of destruction, the most important in regard to otosclerotic bony foci and inner ear fluids.

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This paper is to demonstrate NaF effectiveness through the following seven types of methods: biochemical, experimental, enzymatic, cytochemical, radiological, clinical and by labelling products, which can constitute objective proofs of NaF inhibition action on otospongiotic foci.

I. Biochemical Methods consist in studying NaF action on the proteolytic activity of otospongiotic perilymph samples: 1] by action of the gelatin of photographic films according to Adams's method (1). This screening method has allowed us strong statistical correlations between the intensity of the proteolytic action of the perilymph and the cochlear deterioration: 84% for direct correlations (2,3,4); 2] by microelectrophoresis, leading to specific revelations according to Uriel-Avrameas's method (5,6). This qualitative study has allowed us to find 6 enzymes in perilymph samples, particularly trypsin. The last mentioned has been used as the basic enzyme for our investigations (Figure 1).

it is not possible to give detailed explanations, but we would like to stress the following three essential points:

- 1. NaF has demonstrated its inhibition effect in minute doses: enzymes are inhibited in vitro in the proportion of 0.5 mg NaF to 1 mg trypsin;
- 2. perilymph of control cases (Ménière's and Paget) have given negative results;
- 3. there is an action threshold, under which NaF does not act and beyond which increased doses are not more effective. Awareness of this action threshold is essential in the treatment of the otosponglotic disease by NaF.

Il Experimental Methods

- I. In vitro, G. Shambaugh and S. Kacker (7) have demonstrated by spectrophotometry NaF inhibition action on the hydrolytic action of alphachymotrypsin.
- 2. In vivo, G. Shambaugh has studied calcium increase in long bones, and with Petrovic (8) and Sundar (9) the arrest of bone resorption by NaF. Schatzle and Westerhagen (10,11) observed an important decrease in phosphatasic acid activity in animals treated with NaF. Waltzer (12), Petrovic and Stutzmann (13,14) noted a decrease of phosphatasic acid in the vicinity of otospongiotic foci after extended NaF therapy. Causse (4) demonstrated NaF action on the tryptic activity of the perilymph.
- 3. <u>In laboratory animals</u>, G. Shambaugh and A. Petrovic used tetracycline fluorescence to demonstrate NaF value in inactivation of otosclerotic bony lesions, and in promoting effect of NaF on the calcareous deposit on new bone (15).
- 4. In humans, comparative dosages of trypsin in perilymph samples, before and after NaF therapy, on otosclerotic patients operated on by stapedectomy (4) have shown a constant and significant decrease in trypsin values, averaging 1 to 2 mg/mL after NaF therapy administered during one year at moderate daily doses (15 to 45 mg/day).

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III Enzymatic Methods

Multiple correlations established for four years on 648 samples of perilymph indicate that NaF effectiveness is due to threefold action (2,3) (Figure 2):

- 1. a direct inhibition action on tryptic and similar enzyme values;
- 2. an overall reduction in enzymatic values both in the perilymph and in the microfoci of the otic capsule;
- 3. a direct or indirect conversion of active otospongiotic foci into inactive otosclerotic foci by Ca/P ratio changes (16).

The pattern is precise: trypsin (or a similar enzyme) is the direct cause of cochlear deterioration; α l antitrypsin acts inversely to trypsin; the two major protease inhibitors, α l antitrypsin and α 2 macroglobulin, work together for the inhibition of tryptic activity (4).

IV Cytochemical Proofs

The double-blind study carried out by Paul Bretlau et al (16) on 36 footplates of otosclerotic patients, operated on by stapedectomy, showed that NaF therapy may stabilize otospongiotic lesions by retaining calcium in comparison with phosphorus, thus changing spongiotic types with unstable mineralization, into sclerotic lesions. This means that NaF can convert active foci into inactive ones, either by inactivating the hydrolytic and proteolytic enzymes, and/or by modifying the Ca/P ratio in the foci, the first favoring the second.

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On 12,278 Cases from 1969 through 1980, and allowing a 5 year plus perspective Long-term Cochlear Functional Results

(December 31, 1985)

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V Radiological Methods

As early as 1965, Derlacki and Valvassori (17) used polytomography to determine the prescription of NaF and to evaluate the results. In 1966, Shambaugh (18), by tomography of the otic capsule, studied 46 patients before and after fluoride treatment. In 1969, Valvassori (19), in the same way, exmined 157 patients before and after NaF therapy which lasted 6 months to 2 years. Shambaugh obtained 30% improvement in hearing and in 30% no further deterioration; Valvassori obtained 71% stabilization and 19% improvement after NaF therapy (18,19).

Skeletal X-Ray surveys have been made before and after NaF therapy to detect any increased bone density from various doses of fluoride. None of the cases has shown radiographic changes of fluorosis, and no generalized skeletal effects have been seen corresponding to cochlear capsule changes.

VI Methods Using Labelling Products

In 1973, Strontium 85 was used by Fred Linthicum and Howard House (20) to determine the process of calcium deposition in the footplate of patients with bilateral stapedial otosclerosis before and after NaF administration. The results were compared with calcium deposition in normal canal wall bone. They observed a clear reduction of radio-activity after 6 months administration of NaF. They assert that NaF converts the active otosponglotic phase into and inactive sotosclerotic one, which confirms our work.

VII Clinical Methods

1. Causse et al (21-27) made a computerized study based on a ten year period, from 1969 through 1979, on 12,278 cases, allowing a 5 year or more perspective and an easy evaluation of the situation at any moment thanks to punctual checkings made on the occasion of recent audiometric controls (Figure 3).

The percentage of NaF action is about 80% stabilization results in non-corrected percentages, but these results must be corrected in relation with the 25% spontaneously non-progressive cases. It is obvious that NaF, being an enzymogenesis inhibitor, can only act as a stabilizing factor. Therefore "favorable result" means the arrest of cochlear deterioration in cases with a progression of the cochlear component in stapedial otosclerosis and in cases of pure cochlear otospongiosis.

The corrected results in relation to the control groups and to the 25% spontaneously non-progressive cases, give 73% favorable results for medical cases, and 67% for surgical cases.

2. In children with pure cochlear otospongiosis, results are sometimes surprising, as can be seen from the various audiograms made on an 11 year old child first in 1974, and regularly examined to date. Currently the patient is 23 years old, fully active without hearing aid. Children are the only cases in which such obvious improvement of a sensorineural hearing loss can be observed; it is always irreversible in adults (28-30) (Figure 4).

3. <u>A clinical double-blind placebo-controlled study</u> on the effect of NaF on otospongiotic patients has been carried out by Paul Bretlau et al., in Copenhagen (31). These clinical and audiometric investigations on 95 selected patients with progressive cochlear otospongiosis have shown that the deterioration of hearing is less severe in the group treated with 40 mg NaF than in the placebo-controlled group.

Conclusion

These findings support our concept that NaF is an effective therapy acting on cochlear deterioration in the otospongiotic-otosclerotic disease.

References

- Adams, C.W.M. and Tuqan, N.A.: The Histochemical Demonstration of Protease by a Gelatin Silver Film Substrate. J. Histochem. Cytochem., 9:469-472, 1961.
- Causse, J.R., Uriel, J., Berges, J., Bretlau, P. and Causse, J.B.: Mécanisme enzymatique de l'otospongiose. Action du NaF. Ann. Otol., Paris, 98:269-297, 1981.
- Causse, J.R., Uriel, J., Berges, J., Shambaugh, G.E., Bretlau, P. and Causse, J.B.: The Enzymatic Mechanism of the Otospongiotic Disease and NaF Action on the Enzymatic Balance. Amer. J. of Otology, 3:297-314, 1982.
- 4. Causse, J.R., Uriel, J., Berges, J., Bretlau, P., Shambaugh, C.E. and Causse, J.B.: Objective Changes in Trypsin, Alpha 1 Antitrypsin and Alpha 2 Macroglobulin Values as a Result of Sodium Fluoride Treatment in Patients with otosclerosis. Amer. J. of Otology, 6:38-42, 1985.
- 5. Uriel, J. and Avrameas, S.: Study of Enzyme-Antibody Precipitates, Using Non-chromogenic Substrates. Ann. Biochem., 9:180-182, 1964.
- Causse, J.R. and Chevance, L.G.: Bases biologiques d'un traitement fluoré de l'otospongiose. Résultats expérimentaux et cliniques. Ann. Otol., Paris, 90:139-160, 1973.
- 7. Kacker, S.K. and Shambaugh, G.E.: Effect of Fluoride on Alpha Chymotrypsin. Arch. Otol., 106:260-261, 1980.
- 8. Petrovic, A. and Shambaugh, G.E.: Studies of Fluoride Effects. Acta Otolaryng., 65:120-130, 1968.
- Shambaugh, G.E. and Sundar, V.S.: Experiments and Experiences with Sodium Fluoride for Inactivation of the Otosclerotic Lesion. Laryngoscope, 79:1754-1764, 1969.
- Schatzle, W. and von Westerhagen, B.: Über den Nachweis Saurer Phosphatase im Plexus Cochlearis. Arch. Klin. Ex. Onk. Heilkl., 190:153-165, 1968.
- Schatzle, W. and von Westerhagen, B.: Enzymhistochemisches verhalten des Cortiorgans unter der experimentellen Einwirkung von Natriumfluorid. Arch. Klin. Exp. Onk. Heilki., 100:292-299, 1971.
 Waltzer, G.: Proceedings II Symposium CEMO (Fluoride and Bone), Edit.
- Waltzer, G.: Proceedings II Symposium CEMO (Fluoride and Bone), Edit. Médecine et Hygiène, Geneva, 1978, p. 22-26.
- Petrovic, A. and Stutzmann, J.: Effects of Sodium Fluoride on Osteoformation, Mineralization and Osteoresorption in Otospongiotic and Meatal Bone: a Histomorphometric Study in Labyrinthine Otospongiosis. Proceedings II Symposium CEMO (Fluoride and Bone), Edit. Médecine et Hygiène, Geneva, 1978, p. 109-211.
- 14. Petrovic, A. and Stutzmann, J.: Sequential Analysis of Events in Otospongiotic Mechanism of Action of NaF on Otospongiotic, Meatal and Alveolar Bone. State of the Art Conf. on Labyrinthine Otospongiosis, Lake Bluff, June, 1979.
- 15. Petrovic, A. and Shambaugh, G.E.: Promotion of Bone Calcification by

20

Sodium Fluoride. Short-term Experiments on Newborn Rats Using Tetracycline labeling. Arch. Otol., 83:104-112, 1966.

- Bretlau, P., Hansen, H.J., Causse, J.R. and Causse, J.B.: Otospongiosis: Morphologic and Microchemical Investigation after NaF Treatment. Otoiaryng. Head, Neck Surg., 89:646-650, 1981.
- Derlacki, E.L. and Valvassori, G.E.: Clinical and Radiological Diagnosis of Labyrinthine Otosclerosis. Laryngoscope, 75:1293, 1965.
- Shambaugh, G.E. and Petrovic, A.: The Possible Value of Sodium Fluoride for Inactivation of the Otosclerotic Bone Lesion. Experimental and Clinical Studies. Acta Otolaryng., 63:331-339, 1967.
- 19. Valvassori, G.E.: Cochlear Otosclerosis. Follow-up after Sodium Fluoride Administration. Arch. Otol., 89:383, 1969.
- Linthicum, F.H., House, H.P. and Althaus, S.R.: The Effect of Sodium Fluoride on Otosclerotic Activity as Determined by Strontium. Ann. Otolaryng., 82:609-615, 1973.
- Causse, J.R., Bel, J., Michaux, P., Cezard, R., Tapon, J. and Canut, Y., with the collaboration of M. Desire and D. Massol: Apport de l'informatique dans l'otospongiose — Statistiques sur 15 ans de stapédectomies. Ann. Otol., Paris, 93:149-178, 393-428, 543-576, 1976.
- Causse, J.R. and Causse, J.B.: Clinical Studies on Fluoride in Otospongiosis. Amer. J. of Otology, 6:51-55, 1985.
- Causse, J.R. and Causse, J.B.: Note therapeutique concernant l'utilisation du fluorure de sodium dans l'otospongiose. Les Cahiers d'O.R.L., XV, 5:427-435, 1980.
- 24. Causse, J.R. and Causse, J.B.: Eighteen Year Report on Stapedectomy. Clinical Otolaryng, 5:49-59, 329-337, 397-402, 1980, and 6:67-72, 1981.
- Causse, J.R. and Causse, J.B.: The Rationale of Medical Therapy in Sensorineural Hearing Loss. First Intern. Workshop Otology Today, Riva del Garda (Italy), April, 1982, Otologia Oggi Proceedings, p. 331-346.
- 26. Causse, J.R. and Causse, J.B.: Data Processing in Otosclerosis at the Jean Causse Otology Clinic. Proceedings of the Intern. Conf. on Postoper. Evaluation in Middle Ear Surgery, Scientific Exhibition, Antwerp, 1984, Published in Ars Medici Congress Series, 5:409-414.
- Causse, J.R.: Round-table on Medical Management of Otospongiosis (J.R. Causse, Chairman), Intern. Symposium on Otosclerosis, Lisbon, June 18-20, 1986.
- Causse, J.R. and Causse, J.B.: L'otospongiose, maladie familiale. Sa détection précoce, son traitement médical. Ann. Otol., Paris, 97:325-351, 1980.
- Causse, J.R. and Causse, J.B.: Otospongiosis as a Genetic Disease. Early Detection, Medical Management and Prevention. Amer. J. of Otology, 5:211-223, 1984.
- 30. Causse, J.R. and Causse, J.B.: The Genetics of the Otosponglotic-Otosclerotic Disease. Adv. Audiology, 3:18-31, 1985.
- 31. Bretlau, P., Causse, J.R., Causse, J.B., Hansen, H.J., Johnsen, N.J. and Salomon, G.: Otospongiosis and Sodium Fluoride. A Blind Experimental and Clinical Evaluation of the Effects of Sodium Fluoride Treatment in Patients with Otospongiosis. Annals of ORL, 94:103-107, 1985.

VARIATIONS OF F IN RELATION TO OTHER IONS IN DRINKING WATER

by

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SUMMARY: Ion chromatography was used to measure the variation of some inorganic ions in the drinking water which contained different concentrations of fluoride. As the F ion concentration increased, Na⁺ ion concentration also increased, whereas the concentration of K⁺, Ca²⁺ and M²⁺ ions tended to decrease. The relationship of F⁻ ion concentration to that of such other ions as Cl⁻ and SO₄²⁻ is not clear.

KEY WORDS: Ion chromatography; F, Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄^{2⁻} ions; Relationship of F⁻ ions to other ions in drinking water.

Introduction

Excessive fluoride in drinking water causes chronic toxicity such as mottled teeth. Few reports on the concentrations of fluoride and the other inorganic ions in drinking water, determined by ion chromatography, have appeared in the literature. Because the ion chromatographic analyzer is able to analyze inorganic ions with the same valence simultaneously, it is possible to measure rapidly various inorganic ions under the same conditions.

Here the concentrations of some inorganic ions, in relation to the variation of F^- ions in drinking water, were analyzed by ion chromatography and the results are discussed.

Materials and Methods

64 samples of drinking water were obtained from wells in Saitama and taps used daily by residents. They were collected in plastic bottles after washing with the same water and stored in a cool box as soon as possible. Since the concentration of F^{-} ions ranged from 0.05 µg/mL to 7.40 µg/mL, these samples were divided into three groups (Group A, B and C) in order to make a comparison of ion levels easy.

F levels of samples in group A, B and C ranged from 0.05 μ g/mL to 0.02 μ g/mL, from 0.53 μ g/mL to 0.78 μ g/mL and from 7.20 μ g/mL to 7.40 μ g/mL respectively. The range of F levels and sample number are shown in Table 1. For analysis, the Ion chromatographic analyzer IC 500 (Yokogawa Hokushin Electric, Japan) was used. Analytical conditions for anion and cations are shown in Tables 2 and 3.

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	Table	<u>1</u>		Result	<u>s</u>	
Ra N	nge of F [*] umber of S	ions and amples	The central	results of tions of F	analysis of t ions and th	the con- le other
Group	Range (µg/mL)	Number of Samples	cations shown	in group in Table	A, B and 4, Figures 1	C are and 2
A	7,20-7.40	2	in gro	oup A ave	eraged 7.30	$\mu g/mL$
В	0.53-0.78	8	Concer	ntrations of vere also b	Na ions an	d SO₄² [−] / traces
С	0.05-0.20	54	of K [*]	and Mg ²⁺ i	ons were four	nd.
Chrom	<u>Table 2</u> atographic	2 Conditions	Compa lons in	Fig urison of (Drinking)	<u>pure 1</u> Concentration Water	of F [−]
	for Anic		(µg/m)	L)		
Eluent		10 00 414 No. CO	¹⁰ [
U_004	M NaHCO _a ກ	/0.004M Na ₂ CO ₃			F	
	ume 1	00 vi			·	
Precolumn	4	-60/x 50 mm				
Separator (Column 4	.6 Ø x 250 mm				
	Table	3	5			
Chrom	atographic for Catio	Conditions ons				
Eluent V	alence (+)	0,005M HNO3				
	++) 0.004	•) 0.002M EDA/ IM Tartaric Acid				
Flow Rate	2	mL/min				
Sample Vol	ume 10	0 µL	oL		11111	
Precolumn	4.6	3¢/x 50 mm		А	В	С
Separator C	Column 4.6	iØx 250 mm				

Table 4	
Average Concentrations of Some Inorganic	lons
According to the Variations of F lons	

Group	F ⁻ (µg/mL)	Na ⁺ (µg/mL)	κ ⁺ (µg/mL)	Ca ²⁺ (µg/mL)	Mg ²⁺ (µg/mL)	Cl [⊤] (µg/mL)	SO₄²⁻ (µg/mL)
A	7.30	118	trace	3.0	trace	24.8	116
в	0.60	50.1	1.7	12,9	3,2	20.8	8.2
С	0.13	19,8	3.7	22.6	6.4	31.6	27,4



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In group B, concentrations of F^{-} ions averaged 0.60 µg/mL. Na⁺ ion concentration decreased to half of that of group A; SO₄^{2⁻} ions also decreased markedly. K⁺ and Mg²⁺ ions were detectable; the concentration of Ca²⁺ ions increased to four times that in group A. For chromatogram of group B see Figure 3.

The concentration of f^{*} ions in group C averaged 0.13 µg/mL. Na^{*} ion concentration decreased more than that of groups A or B. The SO₄²⁻ concentration was higher than that of group B, and about one fourth of that of group A. K^{*} and Mg^{2*} ion concentration increased to approximately double that of group B. The concentration of Ca^{2*} ions also increased more than in subjects of groups A or B; the average concentration of Ca^{2*} ions was about seven times that of group A.

Cl ions did not differ appreciably between the three groups.

The correlation between the increase in concentration of F^- and Na⁺ ions (r = 0.78, p < 0.001) was highly positive (Figure 4); SO_4^{-2} ions were also positively correlated (r = 0.60, p < 0.001). On the other

hand, the concentration of Ca^{2^+} , Mg^{2^+} and K^+ ions showed a tendency to decrease according to the increase in F^- ions. As shown in Figure 5, a negative correlation with statistical significance occurred between F^- and Ca^{2^+} ions (r = -0.45, p < 0.001).

Discussion

The electrode method has been commonly used for analysis of F^- ions. However, ion chromatographic analysis developed by Small et al. (1) detects various ions easily and smoothly without any interference of matrix. In this study the relationship between F^- ions and the other inorganic ions in drinking water which contained various concentrations of F^- ions was analyzed by ion chromatography. Na⁺ ions increased according to the increase of F^- ions. The relationship between Na⁺ ions and Cl⁻ ions was known (2); this study confirmed in group C the high positive correlation. However it was difficult to find any relationship between F^- and Cl^- ions (r = -0.03). On the other hand, concentrations of Ca^{2^+} , Mg^{2^+} and K^+ ions decreased with the increase of $F^$ ions. Traces of K^+ ions, especially, are found in drinking water high in $F^$ ions.

Figure 3 Chromatograph of Anions in Group B





No clear increase in $SO_4^{2^-}$ ion concentration along with the increase of F^- ions was found. However, the occurrence of 116 μ g/L of $SO_4^{2^-}$ ions in drinking water high in F^- iosn almost coincided with the data of Liu (3).

Conclusion

These findings show that the concentration of some inorganic ions affect that of others. Therefore, when measuring the concentration of F^{-} ions, concentrations of other inorganic ions should be investigated simultaneously. Ion chromatographic analysis is recommended for investigating drinking water.

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References

- Small, H., Stevens, T.S. and Bauman, W.C.: Novel Ion Exchange Chromatographic Method Using Conductimetric Detection. Anal. Chem., 47:1801-1809, 1975.
- 2. Matsuda, K., Iijima, Y., Tazawa, M. and Takaesu, Y.: Distribution of Fluoride in the Drinking Water in the Suburbs of Hirosaki City in a Dental Fluorosis Area. J. Dent. Health, 28:21-25, 1978.
- Liu, M.: elimination of Excess Fluoride in Drinking Water with Coacervation Induced by Electrolysis Using an Aluminium Electrode. Abstracts: XIV Conference of the International Society for Fluoride Research, Morioka, Japan, 1985, p. 41.

CHANGES IN THE COLLAGEN STRUCTURE OF BONE TISSUE IN EXPERIMENTAL FLUOROSIS

by

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SUMMARY: The changes in the regularity of collagen structure of the corticalis and spongiosa of rat femur and vertebrae, caused by daily intraperitoneal administration of 0.5 mg and 5 mg sodium fluoride, were investigated. Daily administration of 0.5 mg NaF for three months produced a slight, but significant change in the regularity of collagen fibrils; 5 mg NaF/day, a significant decrease in the regularity and disintegration of collagen fibrils. Alteration in the regularity of collagen fibrils is a part of complex disturbances of the fluorotic bone, explained by the toxic effect of fluoride.

KEY WORDS: Experimental fluorosis; Collagen structure; Disorientation of preexisting bone.

Introduction

According to experience with humans, about 10% of the entire preexisting bone tissue is reorganized in the course of one year. This perpetual process of rebuilding and remodeling bone tissue is due to the action of multi-cellular functional units (BMU, BRU or BSU), consisting of osteoclasts and osteoblasts. It is generally accepted that sodium fluoride causes enlargement of the whole bone mass.

The question arises how NaF influences bone tissue, whether enlargement of bone mass is due to increased bone formation (stimulation of osteoblasts) (1-7) and/or to decreased bone absorption (blockade of osteoclasts) (5,8-14). Authors agree that newly-formed is inferior to normal bone, the matrix is irregular (1,7,11,15), the collagen structure of newly-formed bone tissue differs from normal (11), and that mineralization is enhanced (1,3,5,7,11,12).

The aim of our experiments was to investigate the changes of collagen structure in experimental fluorosis.

Material and Methods

Forty-five female rats, each weighing 200 grams, were divided into three groups; 15 animals were given 0.5 mg, 15 received 5.0 mg NaF intraperitoneally, daily, for three months; 15 animals — the control group — received physiological saline solution in the same way.

X ray pictures were taken of the sacrificed animals (Figures 1a, 1b, 1c).

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Figures 1a, 1b, 1c.

Lateral radiograph of controls and rats treated with 0.5 mg NaF. Apparent changes cannot be disclosed compared to controls. Five mg NaF daily for three months caused mainly the enlargement of the lumbar vertebrae. The thickening of corticalis of vertebrae, and formation of spicules are apparent.

Both femurs and the third, fourth and fifth lumbar vertebrae of the animals were investigated histologically, fixed in 10% formaline solution, and decalcified. The decalcifying agent consisted of 24 mL 85% formic acid, 50 mL 35% hydrochloric acid, and 125 mL distilled water. The material was embedded in paraffin, serially sectioned, and stained with picrosirius red (16).

The regularity of collagen fibrils of the preexisting bone tissue was measured by a polarized optic method according to Brace-Kohler in 550 nm monochromatic light using an Opton Standard microscope. Measurements were performed on the corticalis and spongiosa of both femurs and vertebrae using five visual fields in each case; 10 measurements were made in all fields. Average retardation values, characterizing the regularity of collagen fibrils, were calculated. Analysis of significance was performed between the retardation values obtained according to T and Welch (modified T) tests (Figures 1a,1b,1c).

Results

Retardation values, measured in the spongiosa and corticallis of the femurs and vertebrae, are presented in Table 1.

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Retardation of Collagen Fibrils in the Corticalis and Spongiosa of Femurs and Vertebrae

	I	FEMUR	VERTE	BRA
	CORTICALIS	SPONGIOSA	CORTICALIS	SPONGIOSA
Control	0.7083 ±0.0240	0.6780 ±0.0304	0.6901 ±0.0261	0.6878 ±0.0393
0.5 mg NaF	0.6078 ±0.0281	0.5754 ±0.0531	0,6153 ±0,0298	0.5950 ±0.0230
5 mg NaF	0.4390 ±0.0408	0.4284 ±0.0444	0.4212 ±0.0328	0.4275 ±0.0385
Control 2 0.6	910 ±0.0332: 0.5 n	ng NaF Σ 0.5991 ±0	0.0376; 5 mg NaF Σ	0.4275 ±0.0385

The regularity of collagen fibrils in the corticalis and spongiosa of femurs and vertebrae decreased compared to normal (Figure 2a) in rats which received 0.5 mg NaF daily. The observed difference is significant (Figure 2b). On rats which received 5 mg NaF daily, for three months, the regularity of collagen fibrils decreased significantly compared to normal in the corticalis and spongiosa on both femur and vertebrae (Figure 2c).



Figures 2a, 2b, 2c.

Histologic picture of the diaphysis of femur of a control (a) rat treated daily with 0.5 mg NaF (b), and 5.0 mg NaF (c).

- 2a: Well differentiated, lamellar bone.
- 2b: Effect of 0.5 mg NaF collagen structure of preexisting bone tissue slightly disoriented, disintegrated.
- 2c: Significantly disoriented, disintegrated; effect of 5.0 mg NaF.

The intercellular matrix of bone tissue consists of a collagen structure, embedded in proteoglycan aggregates. The process of formation and mineralization of the intercellular matrix are closely related. Isolated injury of any of these components is inconceivable; injury to one of the components is always associated with injury to the others. During the recent investigation irregularity of collagen structure of preexisting bone tissue could be disclosed by a specific topooptic method.

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Conclusion

The investigations disclosed that fluoride causes the regularity of the collagen structure of preexisting, differentiated, lamellar bone to decrease; fluoride exerts its effect not only on the newly generated (newly formed woven) bone tissue, but it also changes the collagen structure of preexisting bone. In our opinion these changes can be considered part of the toxic effect of fluoride exerted on osteocytes. Changes in collagen structure are followed by damage to the matrix (proteoglycan aggregate). We are planning in future to direct our attention to this field of investigation.

References

- 1. Franke, J.: A New Concept of the Effect of Fluorides on Bone. Fluoride, 12:195-208, 1979.
- Franke, J.: Our Experience in the Treatment of Osteoporosis with Relatively Low Sodium-Fluoride Doses. Symposium CEMO, Oct. 9-12, 1977, Nyon. Ed., Médicine et Hygiène, Fluoride and Bone, 1978, p. 256-262.
- 3. Johnson, L.C.: Histogenesis and Mechanism in the Development of Osteofluorosis. In Fluoride Chemistry. Simons, J.H., ed., Acad. Press, New York, London, 4:424-441, 1965.
- 4. Jowsey, J., Riggs, B.L., Kelly, P.J. and Hoffman, D.L.: Effect of Combined therapy with Sodium Fluoride, Vitamin D and Calcium in Osteoporosis. Amer. J. Med., 53:43-49, 1972.
- Petrovic, A. and Stutzmann, J.: Variations of Human Alveolar Bone Turnover as a Function of NaF Concentration in the Drinking Water, Abstracts, Internat. Soc. for Fluoride Research Conf. XI, Dresden, April 8-10, 1981, pp. 40-41.
- 6. Ringe, J-D., Kruse, H-P. and Kuhlencordt, F.: Combined Sodium Fluoride and Calcium Treatment in Primary Osteoporosis. Abstracts, Internat. Soc. for Fluoride Research Conf. XI, Dresden, April 8-10, 1981, p. 44.
- Shupe, J.L., Miner, M.L. and Greenwood, D.A.: Clinical and Pathological Aspects of Fluorine Toxicosis in Cattle. Ann. N.Y. Acad. Sci., 111:618-637, 1964.
- Baylink, D.J. and Bernstein, D.S.: The Effects of Fluoride Therapy on Metabolic Bone Disease. Clinical Orthop. and Related Research, 55:51-85, 1967.
- 9. Bély, M.: Experimental Fluorosis in Rats: NaF Induced Changes of Bone and Bone Marrow. Fluoride, 16:106-111, 1983.
- Goldhaber, P.: The Inhibition of Bone Resorption in Tissue Culture by Non-toxic Concentrations of Sodium Fluoride. Israel J. of Med. Sci., 3:617-626, 1967.
- Krook, L. and Maylin, G.A.: Industrial Fluoride Pollution Chronic Fluoride Poisoning in Cornwall Island Cattle. The Cornell Veterinarian, 69:1-70, 1979.
- Malcolm, A.S. and Storey, E.: Osteofluorosis in the Rabbit: Microradiographic Studies. Pathology, 3:39-51, 1971.
 Rich, C. and Feist, E.: The Action of Fluoride on Bone. In Fluoride in
- Rich, C. and Feist, E.: The Action of Fluoride on Bone. In Fluoride in Medicine. Vischer, T.L., ed., Huber, Bern, Stuttgart, Vienna, 1970, pp. 70-87.
- 14. Spencer, H. and Kramer, L.: Osteoporosis: Calcium, Fluoride and Aluminum Interactions. J. of the Am. Coll. of Nutrition, 4:121-128, 1985.
- Franke, J., Runge, H. and Fingler, F.: Endemic and Industrial Fluorosis. Symposium CEMO, Oct. 9-12, 1977, Nyon. Ed., Médicine et Hygiène, Fluoride and Bone, 1978, pp. 129-143.
- Constantine, V.S. and Mowry, R.W.: Selective Staining of Human Dermal Collagen. II. The Use of Picrosirius Red F3BA with Polarization Microscopy. Invest. Derm., 50:419-423, 1968.

ULTRASTRUCTURAL FINDINGS IN LIVER, KIDNEYS, THYROID GLAND AND CARDIAC MUSCLE OF RABBITS FOLLOWING SODIUM FLUORIDE ADMINISTRATION

by

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SUMMARY: After seven months administration of 10 mg or 50 mg sodium fluoride/day/kg body weight to albino rabbits, their liver, kidneys, thyroid glands and cardiac muscles were studied under transmission electron microscope. Swelling of mitochondria, enlargement of SER cisterns and decrease of RNA granules and RER in these organs were the primary findings. Fluorosis might primarily be an organelle disease which leads to a series of biochemical, pathological and clinical abnormalities.

KEY WORDS: Cardiac muscle; Decrease of RNA granules and RER; Enlargement of SER cisterns; Fluoride intoxication; Kidneys; Liver; Organelle disease; Thyroid gland; Swelling of mitrochondria.

Introduction

The introduction of the electron microscope in medicine has complemented Virchow's "cell pathology" with the new ideas of "organelle pathology." In recent years, reports on structural changes of fluorosis in experimental animals have steadily increased. The present communication presents a comparative study of morphological changes associated with fluoride ingestion. This study has stressed changes of organelles detected in several organs, and has enlightened us on the mechanism of fluorosis.

Materials and Methods

Thirty albino rabbits, ranging in weight from 1 to 1.5 kg were segregated into three groups, with 10 in each. They were fed for seven months under similar conditions. The control group received no sodium fluoride, the remaining groups were fed 50 mg and 10 mg sodium fluoride/day/kg of body weight respectively. After being sacrificed, tissue samples obtained from the liver, kidneys, thyroid gland and cardiac muscle were immediately fixed in 2.5% glutaraldehyde and refrigerated. The samples were prepared for transmission electron microscopy following routine measures, and ultra-thin sections of 400-600 Ångström thickness were examined under a Hitachi 100CX2 transmission electron microscope which operated at 80 kilovolts.

Results

Upon gross and radiological examinations, bones of the fluoride-fed rabbits compared with the controls, demonstrated prominent cortical thickening, hyperostosis and increased bone density, signifying the presence of skeletal fluorosis.

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Liver: Hepatocytes of controls were polygonal in shape, mononucleate or binucleate. Microvilli were noted on the side facing sinusoids. Tight junctions and desmosomes were present between neighboring hepatocytes. Minute bile canaliculi were formed by inward folding of neighboring liver cell membranes. Mitochondria were abundant, polymorphic, with their cristae oriented transversely. Matric granules were numerous and demonstrated high electron density. Golgi complexes, which were situated around the nucleus, endoplastic reticula, glycogen granules and lipid droplets were all discernible.

In fluoride-fed animals, the most prominent change was that numerous mitochondria were swollen and enlarged. Mitochondrial matrix was of low electron density and appeared transparent. Many mitochondrial cristae were broken, with their membrane ruptured or disintegrated (Figure 1). In some areas, the outer and inner mitochondrial membranes were damaged and appeared to be continuous with neighboring RER (Figure 2). Myelin-like inclusions and remnants or membranous structures were occasionally noted in the cytoplasm. RNA granules and RER were reduced in number. Cisterns of SER were enlarged (Figure 3). Lipid droplets were scant.

Kidneys: In the controls, the glomerular basement membrane, podocytes and glomerular capillary loops were discernible. Epithelial cells of proximal con-

Figure 1

Experimental group: mitochondria in hepatocytes swollen, cristae disintegrated, and transparent mitochrondrial matrix, x10000.



Figure 2

Experimental group: mitochondrial membranes in a hepatocyte partially disintegrated, remaining mitochondrial membranes seem to continue with neighboring RER. x29000



Figure 3

Experimental group: in a hepatocyte, RER reduced and cisterns of SER enlarged, x10000



Figure 4

Control group: in an epithelial cell of proximal convoluted tubule, mitochondrial cristae extended almost across the whole mitochondrial diameter. x5800



voluted tubules, which were characterized by their microvilli or brush border at the luminal surface, were rectangular or conical in shape. Mitochondria in the epithelial cells were mostly located at the basal portion close to the basement membrane with their abundant cristae extending nearly across the whole mitochondrial diameter (Figure 4). In these cells, RER were not reduced. Epithelial cells of distal convoluted tubules were cylindrical or rectangular in shape and contained lesser microvilli and mitochondria compared with those of proximal convoluted tubules.

In fluoride-fed animals, mitochondria in podocytes were scant and swollen. The contour of proximal convoluted tubules was intact with their numerous microvilli discernible. Swelling of mitochondria in these cells was prominent. Many mitochondrial cristae were disintegrated. Mitochondrial matrix was transparent and of low electron density. Thickening of basement membrane and reduction of RER were noted in these cells (Figure 5). In epithelial cells of distal convoluted tubules, swelling of mitochondria was not so prominent compared with proximal convoluted tubules.

Thyroid gland: In the controls, follicular epithelial cells were rectangular in shape with multiple microvilli at the follicular surface. They had round

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Figure 5

Experimental group: in epithelial cells of proximal convoluted tubules, mitochondria, prominently swollen, take a spherical shape, with transparent matrix and disintegrated cristae. Basement membrane of cell thickened. x5800



Figure 6

Experimental group: follicular epithelial cells in thyroid gland rectangular in shape, mitochondria swollen, their cristae disintegrated. Cisterns of SER enlarged. x2900



nuclei and cytoplasm containing Golgi complexes, RNA granules, RER and abundant mitochondria. Tight junctions and desmosomes were discernible. Large quantities of phago-lysosomes-like blebs were noted just beneath the apical plasma membrane. Parafollicular C cells were of irregular shape with their cytoplasm containing many organelles, i.e., RNA particles and RER which were of moderate quantity, mitochondria which were of relatively small size, Golgi complexes and others,

In fluoride-fed animals, the most prominent change was swelling of mitochondria in follicular epithelial cells. Mitochondrial cristae were partly disintegrated. Cisterns of SER were enlarged and lysosomes were increased in number (Figures 6,7). In parafollicular C cells, swelling in mitochondria was not prominent.

<u>Myocardium</u>: In the controls, cardiac muscles were slim and elongated, mononucleate, with myofibrils bearing cross striations of dark and light bands, and were connected end to end by intercalated discs composed of desmosomes and tight junctions. Mitochondria were discernible in the sarcoplasm close

Figure 7

Experimental group: Swelling of mitochondria in thyroid gland, their cristae disintegrated, enlargement of SER cisterns in follicular epithelial cells. x5800



Figure 8

Control group: normal mitochondria with long and closely packed cristae in cardiac muscle cells, x14000



to the nuclei, having long and closely packed cristae (Figure 8). Glycogen granules and lipid droplets were noted around mitochondria.

In fluoride-fed animals, organelles were essentially normal even in the higher dosage group. No prominent changes were noted in mitochondria except that disintegration and formation of lamellated myelin-like figures were found occasionally (Figures 9,10).

Discussion

The primary function of mitochondria is to provide readily available energy for living activity needs. This process is coupled to oxidation of nutrient substrates and is catalyzed by a series of enzymes located in the mitochondria. The morphology of mitochondria alters in accordance with change in metabolic state and with pathological processes. Swelling and disintegration of mitochondria (as noted by the authors in various organs of fluoride-fed animals) indicate structural changes of inner mitochondrial membrane, which in turn lead to a loss of ATP-synthesizing ability, supression of ATP-production, and a failure of ATP-dependent functions - for example, functioning of the sodium pump at the plasma membrane. Enlargement of mitochondria in fluoride-fed animals is believed to be a compensatory process due to ATP deficiency. Myelin-like

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Figure 9

Experimental group: no significant changes in mitochondria of cardiac muscle cells, x29000



Figure 10

Experimental group: a myelin-like figure located at one side of a mitochondrion of a cardiac muscle cell. x29000.



figures indicate degeneration and disintegration of mitochondria. Enlargement of SER cisterns in these animals signifies intensification of the detoxication process. Reduction in the number of RER and RNA granules is suggestive of supression of protein synthesis, whereas an increase in number of lipid droplets indicates disturbances of fat metabolism.

Fluoride-induced damage to liver cells has been reported by Lavrushenko et al (1). These authors found swelling and fragmentation of mitochondria, breakdown of mitochondrial membranes, and degranulation suggestive of supression of protein synthesis. The present communication substantiates these authors' observations.

Concerning the morphological changes in kidneys in fluorotic animals, Kour et al (2) reported light microscopic findings, including atrophy of glomeruli, edema and necrosis of tubular cells, nuclear degeneration and pyknosis, as well as complete disintegration of renal tissue. The changes noted in the present study are not as severe. Under light microscopy, the only finding in our animals was thickening of tubular basement membrane. Although we did find a series of changes in podocytes and epithelial cells of proximal convoluted tubules under electron microscope, no significant changes in epithelial cells of distal convoluted tubules were found. These facts suggest that, in fluoride intoxication, epithelial cells of proximal convoluted tubules are selectively damaged because they function differently from distal convoluted tubules.

In 1971, Sundstrom (3) reported swelling of endoplasmic reticula in parafollicular C cells in thyroid gland of fluorotic rats. In our animals, swelling of mitochondria with disintegration of their cristae in epithelial follicular cells indicated disturbance of oxidative phosphorylation. However, we found no significant changes in parafollicular C cells. Cardiac muscle cells also showed no significant changes.

In the presence of toxic levels of fluoride in the blood stream, many organelles, especially mitochrondia, were damaged. Morphological changes developed to various extent in some organs and organelles, indicative of other factors which play a role in the development of morphological changes. These morphological changes will inevitably lead to a series of biochemical, pathological and clinical abnormalities, such as supression of enzymes namely acid and alkaline phosphatase (4), and others.

Conclusion

The present study suggests that fluoride intoxication is primarily an organelle disease.

References

- Levrushenko, L.F., Trunov, V.I., Okunev, V.N.: Morphofunctional Characterization of Certain Ultrastructures of Rat Liver Hepatocytes under the Prolonged Influence of Sodium Fluoride. Tsilogia i Genitika, 14:19-20, 1980.
- Kour K. and Singh, J.: Histological Findings in Kidneys of Mice Following Sodium Fluoride Administration. Fluoride, 13:161-167, 1980.
- Sunderstrom, B.: Brief Report Thyroid C Cells on Short Term Experimental Fluorosis in the Rat. Acta Path. Microbil. Scand., Section A, 79:407-408, 1971.
- Bogin, E., Abramo, M., Avider, Y. and Israeli, B.: Effect of Fluoride on Enzymes from Serum, Liver, Kidney, Skeletal and Heart Muscles of Mice. Fluoride, 9:42-46, 1976.

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ENDEMIC SKELETAL FLUOROSIS CLINICAL AND RADIOLOGICAL VARIANTS (Review of 25 Years of Personal Research)

by

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Our continuing studies over the past 25 years have shown that endemic skeletal fluorosis can be present in various clinical and radiological forms (1-22). The purpose of this communication is to emphasize the clinician's responsibility, when treating patients with bone and joint disorders, to consider the diagnosis of skeletal fluorosis, particularly when the patient's symptoms are unusual (2,6,19).

All the 5,600 patients in our study had been living in endemic fluorosis areas since birth; they had consumed water with a natural fluoride content of 1.5-25 ppm. Nutritional status and dietary intakes were assessed in each patient. The diagnosis of skeletal fluorosis was confirmed by radiological generalized osteosclerosis, periosteal bone formation and calcification of inter-osseous membranes (6,9,17), and the finding of high concentrations of fluoride in drinking water, plasma and urine. Raised levels of plasma alkaline phosphatase and parathyroid hormone supported the diagnosis of associated rickets, osteomalacia and secondary hyperparathyroidism (1,3-5,7,10,17).

Histopathology and histomorphometry of iliac crest bone biopsy and analysis of bone ash for fluoride content were performed only when diagnosis was uncertain. All such tests were performed using methods previously reported (4,5,19).

Clinical and radiological variants of endemic skeletal fluorosis are summarized in Tables 1 and 2 and Figures 1-4.

Classical clinical features characteristic of skeletal fluorosis include arthralgia, backpain, stiffness, rigidity, limitation of movements and inability to close the fists. Patients are often mistakenly diagnosed as rheumatism, cervical spondylitis, osteoarthritis, renal osteodystrophy, renal hyperparathyroidism, osteopetrosis and metabolic bone disease (rickets, osteomalacia, osteoporosis, hyperparathyroidism).

Recently we also identified an endemic disorder of bone disease and deformities consistent with multiple epiphyseal dysplasia which, due to the clinical similarity, is often mistakenly diagnosed as skeletal fluorosis. The majority of these individuals showed autosomal dominant inheritance, were short in stature, had limping gait, pain in hips and knees, constricted hip flexion, lumbar lordosis, genu valgum, genu varum and leg rotation. Though clinical and radiological signs were very heterogeneous, most of these cases could be grouped into (a) spondylo-epiphyseal dysplasia (with platyspondyly) and

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Table 1

Clinical Variants of Endemic Skeletal Fluorosis

Adults

- A. Asymptomatic (Radiological skeletal fluorosis)
- B. Symptomatic
 - 1. Vague pains, aches, arthralgia
 - +Backpain, pain in cervical spine, stiffness, rigidity, constipation.
 - +Limitation of movements at joints, inability to close fists.
 - +Difficulty in walking, generalized attitude of flexion, ankylosis at spine, hips, knees and elbows.
 - +Inability to walk, extreme fixity of chest and spine, crippled.
 - 1.5 +Rarely neurological complications (radicular pains, weakness and wasting of muscles, acroparesthesiae), cord compression (paraplegia, quadriplegia).

Children

 A. Asymptomatic (Radiological skeletal fluorosis)

- Congenital, fetal or neonatal (caused by transplacental transport of fluoride during pregnancy in women residing in endemic areas).
- 2. Acquired (due to chronic ingestion of fluoride in drinking water).
- B. Symptomatic
 - 1. Vague pains and aches, arthralgia
 - 1.1 +Stiffness and inability to close fists.
 - 1.2 +Rigidity and flexion at cervical and lumbar spine.
 - 1.3 +Deformities Coxa vara, genu varum, genu valgum, bowing of legs, wind-shift deformity, chest deformity (more particularly observed in children with dietary calcium deficiency).
 - 1.4 +Flexion (ankylosis) at spine, hips, knees and elbows.
 - 1.5 Crippling with generalized forward flexion, ankylosis of joints, fixity of chest and spine.

Table 2

Radiological Variants of Skeletal Fluorosis

Adults

- 1. Generalized osteosclerosis
- 1.1 +Periosteal bone formation most commonly seen at elbows, dense and thick cortex, diminished medullary cavity.
- 1.2 +Calcification of interosseous membrane and ligaments.
- 1.3 +Osteophytosis, exostoses, calcifications of tendons, capsules and muscular attachments.
- 1.4 +Metabolic bone disease (osteomalacia, osteoporosis and secondary hyperparathyroidism).

Children

- 1. Generalized osteosclerosis
- 1.2 +Osteoporosis, periosteal bone formation and coarse trabeculations most commonly observed at elbows and knees, calcification of ligaments.
- 1.3 +Metabolic bone disease (rickets, osteoporosis and secondary hyperparathyroidism).
- 1.4 +Calcification of interosseous membrane (rarely seen under 15 years of age).

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Figure 1

Figure 2

Clinical picture of 18 year old patient of endemic skeletal fluorosis showing generalized attitude of flexion with ankylosis at cervical spine, hips, knees and elbow. Photograph showing genu valgum with external rotation and bending of bones of legs in a 25 year old patient of endemic skeletal fluorosis.

(b) multiple epiphyseal dysplasia (with minimal or no spinal involvement). We have named this rare disorder of bone development as Handigodu syndrome of multiple epiphyseal dysplasia and its variants because Handigodu is the name of the village where this disease was first observed.

Fluoride toxicity afflicts children more severely and after a shorter exposure to fluoride than adults, due to the greater and faster accumulation of fluoride in the metabolically more active growing bones of children (15). Typical clinical features of skeletal fluorosis in children include vague pains and aches, arthralgia and stiffness at the cervical and lumbar spine and inability to close their fists. Children with inadequate intake of dietary calcium and vitamin D foten show additional unusual features of the disease (Table 1). Calcification of interosseous membranes in children is rare, usually occurring only after 15 years of continuous exposure.

Skeletal fluorosis was observed in the newborn whose mothers resided in areas where drinking water fluoride intake reached 20-50 mg/day. Such congenital fluorosis results from maternal fluoride transport across the placenta (11,12).



Figure 3

Radiograph of the hand showing osteosclerosis, modelling defects in the metacarpals and phalanges and resorption of the cancellous bone with thinning of the cortices in a five month old child of endemic skeletal fluorosis.

Figure 4

Radiograph of pelvis of 35 year old female showing osteosclerosis, deformed pelvic cavity and trabeculations suggestive of osteomalacia and hyperparathyroidism secondary to fluorosis.



Volume 21, No. 1 January, 1988 Dental fluorosis occurs in children who are exposed to high fluoride intake before completion of dental mineralization. The incidence and severity of dental fluorosis and caries increased in children who had been drinking water the fluoride content of which is more than I ppm. Typical radiological findings of dental sclerosis and condensation of alveolar bone around the roots of the teeth were seen only in children with adequate nutrition. Resorption of alveolar bone and loss of lamina dura were more severe and frequent in children with endemic skeletal fluorosis having dietary calcium deficiency.

Various factors influencing the course and severity of skeletal fluorosis include the following: (1) drinking water fluoride concentration, (2) total daily fluoride intake, (3) duration of fluoride ingestion, (4) fluctuations in fluoride intake, (5) solubility of ingested fluoride, (6) age at the time of fluoride ingestion, (7) nutritional status, (8) individual biological response, (9) species effect, (10) stress factors, (11) complexes with other mineral and trace elements, (12) climatic factors, (13) alkalinity and hardness of drinking water, (14) pH of gastrointestinal tract and, (15) renal function. The most important factors, however, were drinking water fluoride concentration, total daily fluoride intake, duration of exposure to fluoride and dietary intake of calcium and vitamin D. The interaction of fluoride and parathyroid hormone in calcium deficient children was a major factor in the production of genu valgum and varum deformities in this age group.

Conclusion

Endemic skeletal fluorosis is a syndrome of bone disease and deformities caused by chronic ingestion of fluoride through drinking water. Neonatal skeletal fluorosis as well as clinical and radiological variants of skeletal fluorosis must be carefully differentiated from other bone and joint disorders. Interaction of fluoride with calcium, trace elements and parathyroid hormone is relevant in the causation of fluorosis variants. Diagnosis of skeletal fluorosis is based on the radiographic findings of osteosclerosis, periosteal bone formation and interosseous membrane calcification, and increased levels of fluoride in water, plasma, urine and bone. Raised plasma alkaline phosphatase and parathormone levels support the diagnosis of associated metabolic disease and hyperparathyroidism secondary to fluorosis. Bone histomorphometry is not diagnostic.

Our continuing identification of new endemic areas in India suggests that the total population that may need protection against fluorosis may be around 10 million. Emerging evidence of new clinical and radiological syndromes of fluorosis requires greater attention and clinical acumen to distinguish these conditions from other known disorders of bone and mineral metabolism. The purpose of this communication will have been accomplished when clinicians consider and evaluate the possibility of skeletal fluorosis in every patient with bone and joint disease, particularly in countries with high fluoride in the drinking water.

References

- Teotia, S.P.S., Teotia, M. and Kunwar, K.B.: Some Metabolic Studies in Skeletal Fluorosis with a New Approach to Its Treatment. Fluoride, 2:142-142, 1969.
- 2. Teotia, S.P.S., Teotia, M. and Kunwar, K.B.: Endemic Skeletal Fluorosis. Arch. Dis. Child., 46:686-691, 1971.
- 3. Teotia, S.P.S. and Teotia, M.:Hyperactivity of Parathyroid Glands in Endemic Osteoporosis. Fluoride, 5:115-125, 1972.

- 4. Teotia, S.P.S. and Teotia, M.: Secondary Hyperparathyroidism in Patients with Endemic Skeletal Fluorosis. Brit. Med. J., 1:637-640, 1973.
- Teotia, S.P.S. and Teotia, M.: Endemic Skeletal Fluorosis in Children: Evidence of Secondary Hyperparathyroidism. In: Clinical Aspects of Metabolic Bone Disease, Frame, B., Parfitt, A.M. and Duncan, H., eds., Exerpta Medica. Amsterdam, 1973, pp. 232-238.
- Teotia, S.P.S., Teotia, M., Rohatgi, V.K. and Teotia, N.P.S.: Endemic Skeletal Fluorosis and Metabolic Bone Disease (A Radiological and Morphometrical Study of Bone). J. Indian Medical Assoc., 63:207-211, 1974.
- 7. Faccini, J.M. and Teotia, S.P.S.: Histopathological Assessment of Endemic Skeletal Fluorosis. Calc. Tiss. Res., 16:45-57, 1974.
- 8. Teotia, S.P.S., Teotia, M. and Teotia, N.P.S.: Joints. Fluoride, 9:19-24, 1976.
- 9. Teotia, S.P.S., Teotia, M. and Teotia, N.P.S.: Skeletal Fluorosis: Roentgenological and Histopathological Study. Fluoride, 9:91-98, 1976.
- Teotia, S.P.S., Teotia, M., Singh, R.K., Taves, D.R. and Heels, Susan: Endocrine Aspects of Endemic Skeletal Fluorosis, J. Assoc. Phys. India, 26:995-1000, 1978.
- 11. Teotia, S.P.S. and Teotia, M.: Maternal Fluoride Metabolism: Its Effects on the Foetus. Bulletin, International Pediatric Association, 3:42-44, 1979.
- 12. Teotia, S.P.S. and Teotia, M.: Metabolism of Fluoride in Pregnant Women Residing in Endemic Fluorosis Areas. Fluoride, 12:58-64, 1979.
- Teotia, M. and Teotia, S.P.S.: Advances in the Understanding of the Effect of Fluoride on Bone. Proceedings, XI Conference of the International Society for Fluoride Research, Dresden, GDR, 1981, pp. 6-8.
- Teotia, S.P.S., Teotia, M., Singh, D.P.: Technical Report on Metabolism of Fluoride in the Newborn, Infants and Children Living in Endemic Fluorosis Areas. Department of Environment, Government of India, New Delhi, 1982, pp. 1-54.
- Teotia, S.P.S., Teotia, M. and Singh, D.P.: Fluoride and Calcium Interactions. Syndromes of Bone Disease and Deformities (Human Studies). Proceedings, International Symposium: Disorders of Bone and Mineral Metabolism. Henry Ford Hospital, Michigan, USA, 1983, pp. 502-503.
- Teotia, M., Teotia, S.P.S., Singh, D.P. and Singh, C.V.: Chronic Ingestion of Natural Fluoride and Endemic Bladder Stone Disease. Indian Pediatrics, 20:637-642, 1983.
- Teotia, S.P.S. and Teotia, M.: Fluorosis-India: Advances in the Understanding and Prevention of Endemic Fluoride Toxicity. Proceedings, International Development Research Centre (IDRC) Canada Sponsored Project, 1983, pp. 1-58.
- Teotia, S.P.S., Teotia, M., Singh, D.P. and Singh, N.P.: Endemic Fluorosis

 Change to Deeper Bore Wells as a Practical Community-acceptable
 Approach to Its Eradication. Fluoride, 17:48-52, 1984.
- Teotia, S.P.S., Teotia, M., Singh, D.P., Anand, Vandana, Singh, C.V. and Tomar, N.P.S.: Environmental Fluoride and Metabolic Bone Disease - An Epidemiological Study (Fluoride and Nutrition Interactions). Fluoride, 17:14-22, 1984.
- 20. Teotia, S.P.S. and Teotia, M.: Endemic Fluorosis in India: A Challenging National Health Problem. J. Assoc. Phys. India, 32:347-352, 1984.
- Teotia, S.P.S., Teotia, M. and Singh, D.P.: Bone Static and Dynamic Histomorphometry in Endemic Skeletal Fluorosis. In: Fluoride Research 1985, Studies in Environmental Science, Tsunoda, H. and Yu, M.-H., eds., Volume 27, 1986, pp. 347-355.
- Teotia, S.P.S., Teotia, M., Singh, D.P. and Nath, M.: Deep Bore Drinking Water: A Practical Approach in the Eradication of Endemic Fluorosis in India. Ind. J. Med. Res., 85:669-673, 1987.

PARATHYROID GLANDS, CALCIUM AND VITAMIN D IN EXPERIMENTAL FLUOROSIS IN PIGS

by

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(Abstracted from Calcif. Tissue International, 38:222-226, 1986)

Eight experimental pigs which received 2 mg F/kg b.w. per day from age 8-14 months were compared with eight controls. Concentrations of plasma fluoride and total plasma calcium were assessed at intervals throughout the experiment. At the same time, concentrations of immunoreactive parathyroid hormone were measured by a homologous labelled antibody for porcine hormone; a radioimmunoassay was used to assess concentrations of 1.25-DHCC and 24.25-DHCC. Parathyroid tissue volumes were assessed at the end of the experiment by quantitative histology using volumetry and point counting. Plasma fluoride increased from 0.0007 ± 0.0001 mmol/liter to 0.0127 ± 0.002 mmol/liter in pigs receiving fluoride. Total plasma calcium remained the same throughout the experiment. Volumes of parathyroid hormone 1.25-DHCC and 24.25-DHCC, were not significantly changed.

Conclusion: Disturbance of calcium homeostasis is not an obligatory finding in dental and skeletal fluorosis.

- KEY WORDS: Dental, skeletal, fluorosis; Parathyroid hormone; Pigs; Plasma Calcium; Plasma fluoride.
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THE HASTINGS FLUORIDATION EXPERIMENT: SCIENCE OR SWINDLE?

by

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(Abstracted from The Ecologist 16:243-248, 1986)

The Hastings fluoridation study in New Zealand, 1954-1970, is listed in textbooks throughout the world as an important study confirming the effectiveness of water fluoridation. It is claimed that "free smooth-surface caries was reduced by 87 percent . . . approximal caries by 73 percent . . . and occlusal surface caries by 39 percent . . .," the greatest reductions being among

*A Critical-Historical Reassessment of the Hastings Fluoridation Experiment

6-year-olds: 74 percent by 1961 and 87 percent by 1964. However, most of these had occurred in the first few years of the project; 42 percent by 1957 and 61 percent by 1959. Such spectactular reductions led to acceptance of widespread fluoridation in New Zealand.

The Hastings-Napier investigation was first described as an "experiment." The neighboring town of Napier was considered an ideal control because it used essentially the same ground water (unfluoridated: 0.15 ppm). However, in 1957, the city of Napier was abandoned as a control. The project was subsequently called a "before-and-after demonstration;" 58% less decay had been recorded in Napier (unfluoridated) than in fluoridated Hastings.

The 1982 New Zealand Official Information Act permitted public access to government archives. Thereby a considerable amount of information not in agreement with the currently accepted published version of the Hastings fluoridation study was revealed; namely, 1) the claimed reductions in decay, which were greatest for younger children, were brought about partly, if not mainly, by a local change in diagnostic procedure following the introduction of fluoridation; 2) reductions over such short periods are, by today's statistical standards, beyond the "limit of credibility" for genuine decay reductions; 3) a reduction in dental decay occurred in other, non-fluoridated places throughout New Zealand during the time of the study, making it difficult for public health officials to present convincing statistics showing that the claimed reductions were related to fluoridation. The reduction occurred in the control town as elsewhere.

The Hastings experiment had commenced in 1952. Pre-fluoridation dental examinations of Hastings children were not only not published but they had been destroyed in one of the department's "periodical purges of records." The experiment was carried out by Dr. T.G. Ludwig who replaced Dr. R.E.T. Hewat, Dental Research Officer of the New Zealand Medical Research Council. Hewat had doubts whether the benefit claimed to result from this measure is fully supported by scientific evidence. "In New Zealand," Dr. Hewat stated, "we have found that many factors are interrelated with the carles rate, and I am not aware that any consideration has been given to such influences in the published data on carles and fluorine."

Thus the reported reductions were, at least partly if not wholly, the result of factors other than fluoridation. This fact, although known to those responsible for the study, was never mentioned in the official and scientific reports of it. The study was, it seems, more a public relations exercise than a scientific one. Nonetheless, it is still being cited in dental scientific literature, and in textbooks as being the latter.

KEY WORDS: Carles criteria, Fluoridation, Hastings experiment.

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FLUORIDE, ALTITUDE AND DENTAL FLUOROSIS

by

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(Abstracted from Caries Res. 20:473-480, 1986)

Children aged 11-15 years from three low-fluoride zones (< 0.5 ppm in drinking water), situated at sea level, 1,500 m and 2,400 m above sea level, and from two higher-fluoride zones (0.5-1.0 ppm in drinking water) were examined for dental fluorosis (see Table). In the low-F zones, 36.4% of the children at sea level had dental fluorosis, compared to 78.0% at 1,500 m, and 100.0% at 2,400 m. In the higher-F zones 71.2% had dental fluorosis at sea levels compared to 93.8% at 1,500 m (p < 0.001). The severity of dental fluorosis of each tooth type increased significantly with increase in altitude in both low and higher-fluoride zones (p < 0.001).

This study indicates for the first time that populations living at high altitudes may be more susceptible to dental fluorosis than those at low altitudes for a given concentration of fluoride in drinking water. In all five populations, the intra-oral distribution of dental fluorosis showed a similar pattern; the prevalence was lowest in incisors and first molars; highest in premolars and second molars. Pitting of enamel (TFI \geq 5) in the low-F samples hardly occurred at sea level, was more pronounced in the 1,500-meter sample, and marked at 2,400 m. In the 1,500-meter sample, pitting occurred most frequently in the first molars and premolars; in the 2,400-meter sample these teeth as well as the canines and upper incisors also exhibited a relatively high proportion with pitting.

In the lower-fluoride zones, the prevalence and severity of dental fluorosis of each tooth type was significantly higher in the 2,400-meter area than in the 1,400-meter area, which in turn was significantly higher than in the sea level area (conditional chi square: p < 0.001). The proportion of teeth and the proportion of individuals affected at each level was highest among the 2,400-meter sample and lowest among the sea level sample.

According to this study, temperature and prevalence and severity of dental fluorosis are inversely related. Whereas temperature may have some influence, that of altitude is much greater.

Table

Percent of Dental Fluorosis Related to Level of F in Water and Altitude

F in Water	Sea Level	1500 Feet	2400 Feet
0.5 ppm	36.4%	78%	100%
0.5-1.0 ppm	71.2%	93.8%	

At all F levels severity of dental fluorosis increased significantly with increase in altitude.

In an experimental study on rats, Angmar-Mansson et al. concluded that chronic hypobaric hypoxia can produce changes in incisor enamel mineralization that resemble fluorosis even on diets with very low fluoride concentrations. The present study shows that differences in prevalence and severity at the same altitude are associated with fluoride concentrations in drinking waters, whereas at the same time in areas with similar levels of fluoride concentrations, increases in altitude are associated with an increase in the prevalence and severity of enamel changes. In all five populations examined here the intra-oral distribution of enamel changes was similar to that which has been reported for dental fluorosis by Larsen et al. Disturbances of the enamel must be considered to be fluoride-induced. The present evidence indicates that altitude causes physiological changes which affect the way in which fluoride is handled by the body. Consequently the toxic effects of fluoride upon the enamel may be exacerbated.

Further studies are in progress to elucidate both the relative contribution of different sources of fluoride and the dietary patterns which may affect the bio-availability of fluoride from such sources.

KEY WORDS: Altitude; Dental fluorosis; Epidemiology; Fluoride.

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THERAPY FOR OSTEOPOROSIS: CHARACTERIZATION OF THE SKELETAL RESPONSE BY SERIAL MEASUREMENTS OF SERUM ALKALINE PHOSPHATASE ACTIVITY

bу

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(Abstracted from Metabolism, 36:211-218, 1987)

Serial measurements of serum alkaline phosphatase activity (SALP) in 53 osteoporotics treated with 66 to 110 mg of sodium fluoride (NaF) for 12 to 91 months showed that SALP increased in 87% of the subjects during therapy with fluoride.

Since most osteoporotics show increased SALP in response to fluoride therapy, although the extent of the response is quite variable, measurements of alkaline phosphatase activity in serum may serve as a valuable tool for convenient monitoring of skeletal response to fluoride.

Thirteen of 53 ambulatory patients (23 males and 30 females) were treated with fluoride only. SALP was significantly increased after one and two years

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of fluoride therapy. Serum phosphorus was unchanged. A small, yet statistically significant, decrease in serum calcium occurred after two years of treatment. The decrease in serum calcium was not related to the increase in SALP (r = .08).

The onset for symptomatic response to fluoride therapy in the 38 patients ranged from four to 85 months (mean \pm SD, 20 \pm 16 months).

Large interpatient variations were observed in all aspects of the SALP response to fluoride (namely, time to first significant increase, rate of increase and peak increase in SALP) and similar interpatient variations in the onset of radiographic and symptomatic improvements. These variations in skeletal response to fluoride therapy could not be explained by differences in age, sex, osteoporosis etiology, fluoride dose or inclusion of other drugs. However, at least part of the variable SALP response to fluoride was related to variation in pre-treatment SALP.

Further studies will be necessary to identify the source(s) of the variations in the skeletal response in fluoride. In this regard, the application of serial SALP measurements as an index of skeletal response to fluoride therapy may prove useful.

If the patient does not show an increase in SALP after two years of therapy with an adequate blood level of fluoride, the patient is considered to be a poor responder to fluoride therapy for osteoporosis.

- KEY WORDS: Fluoride therapy; Osteoporosis treatment; Serum alkaline phosphatase; Skeletal response to fluoride.
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TRENDS IN THE PREVALENCE OF DENTAL FLUOROSIS IN THE UNITED STATES: A REVIEW

by

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(Abstracted from J. of Public Health Dentistry 47:71-79, 1987)

The purpose of this paper is to determine whether an increase in dental fluorosis has occurred since Dean's time among children residing primarily in areas where the level of fluoride in water is around 1 ppm or less. Serious study of dental fluorosis, a hypoplasia of the dental enamel caused by consumption of excess fluoride during the years of tooth calcification, began

with McKay in the early part of the century: It was referred to, at that time, as Colorado Brown Stain.

Prior to 1940, water and food were probably the main sources of fluoride exposure for most individuals. Considering the multiple sources of fluoride now available, topical fluorides, dietary fluoride supplements, as well as fluoridated dentifrices and mouthrinses, and the possible increase in environmental levels of fluoride, an increase in the prevalence of dental fluorosis should not be surprising. Oldak and Leverett reported that 22 percent of first and second grade children living in a nonfluoridated area but less than 1 percent had fluorosis in the primary teeth. Leverett found that the prevalence of dental fluorosis was 3.5 times higher in nonfluoridated communities and two times higher in fluoridated communities than would be expected based on Dean's findings in 1942. In Auckland, New Zealand, Cutress et al. found that diffuse enamel opacities occurred more frequently in fluoridated than nonfluoridated areas (p < 0.001), with a mouth prevalence of 19 percent in the fluoridated areas, but only 8 percent in nonfluoridated areas.

Assenden and Peebles who used Moller's modified version of Dean's index, found a prevalence of fluorosis in the group receiving fluoridated water, of 32.6 percent, considerably higher than the 10 percent prevalence suggested by Dean to occur in fluoridated areas.

Continued study and monitoring of dental fluorosis in fluoridated and nonfluoridated communities is recommended in view of the multitude of fluoride sources available today.

KEY WORDS: Dental fluorosis; Fluoridation; Fluoride ingestion

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INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (January, April, July, October). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published in Fluoride. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

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1. General – No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system (SI).

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